

Cell Chemical Biology, Volume 25

Supplemental Information

Epidithiodiketopiperazines Inhibit Protein

Degradation by Targeting

Proteasome Deubiquitinase Rpn11

Jing Li, Yaru Zhang, Bruno Da Silva Sil Dos Santos, Feng Wang, Yuyong Ma, Christian Perez, Yanling Yang, Junmin Peng, Seth M. Cohen, Tsui-Fen Chou, Stephen T. Hilton, and Raymond J. Deshaies

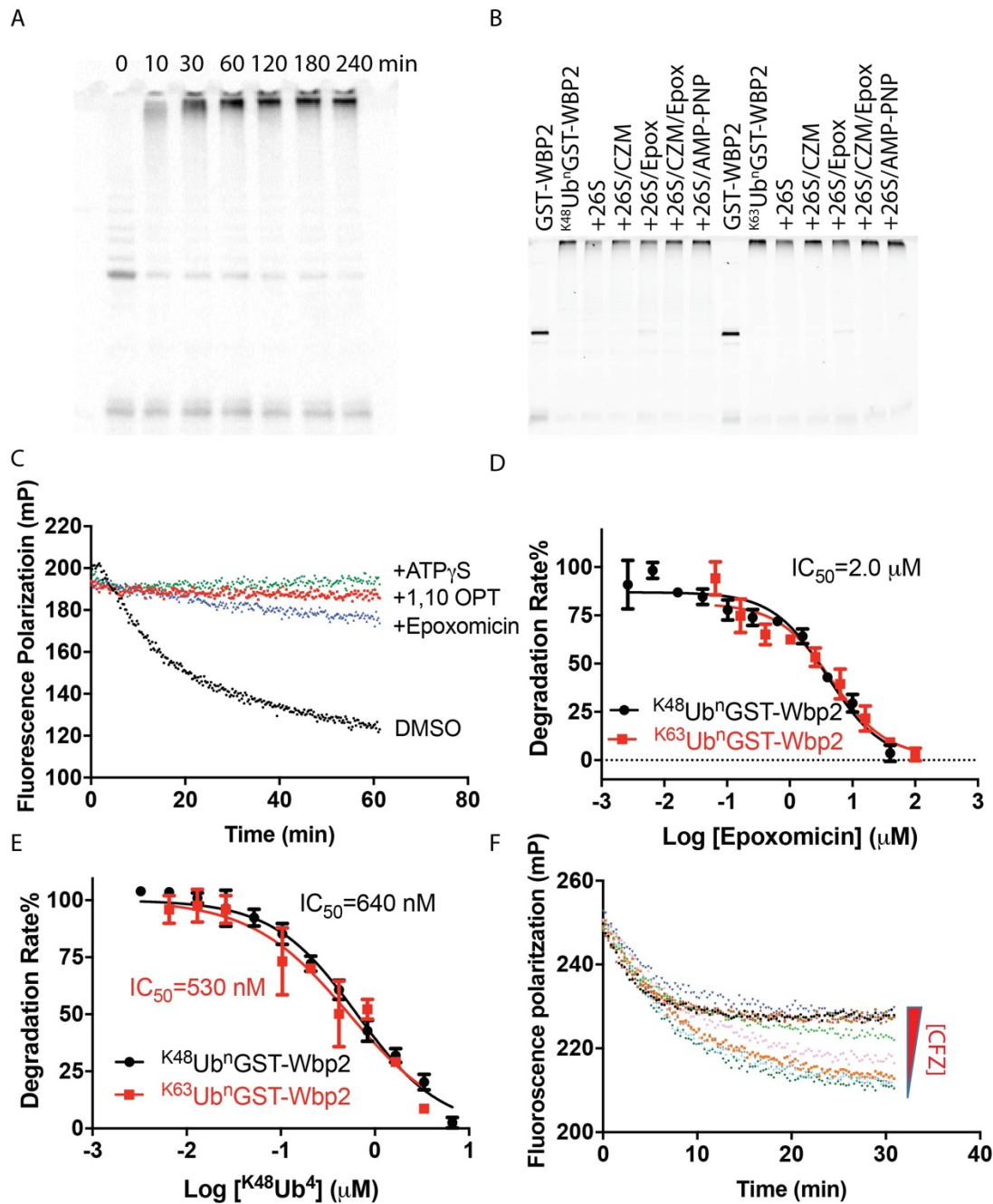


Figure S1. Development of an assay to monitor protein degradation. Related to **Figure 1** (A) *in vitro* ubiquitination of GST-Wbp2 with Ube1, UbcH5a and Rsp5-E6AP at room temperature. Reactions were fractionated by SDS-PAGE and analyzed using a Typhoon fluorescence scanner at different time points as indicated. (B) Proteasomal degradation of UbⁿGST-Wbp2. 20 nM K48UbⁿGST-Wbp2 was incubated with 2.5 nM 26S proteasome at 37°C for 2 hours in the absence and presence of different inhibitors (20 μ M CZM or 5 μ M epoxomicin/Epox or 1 mM AMP-PNP). Reactions were fractionated by SDS-PAGE and analyzed using a Typhoon fluorescence scanner. (C) Measurement of proteasome activity using fluorescence polarization assay. K48UbⁿGST-Wbp2 (2.5 nM) was incubated with 1 nM 26S proteasome at 37 °C in the absence or presence of different inhibitors (10 μ M Epox or 2 mM ATP γ S or 2 mM 1,10-phenanthroline (1,10-PT)). (D) Epoxomicin inhibits the degradation of the Wbp2 substrate. Shown is the dose-response curve of proteasome activity measured at 37 °C using UbⁿGST-Wbp2 as substrate and proteasome with different concentrations of epoxomicin. (E) Shown are the dose-response curves of proteasome activity measured at 37°C using UbⁿGST-Wbp2 as substrate and proteasome in the presence of different concentrations of K48-linked Ub4. (F) Shown is the reaction kinetics measured at 37 °C using K63UbⁿGST-Wbp2 as substrate and lysate from cells treated with different concentrations of carfilzomib.

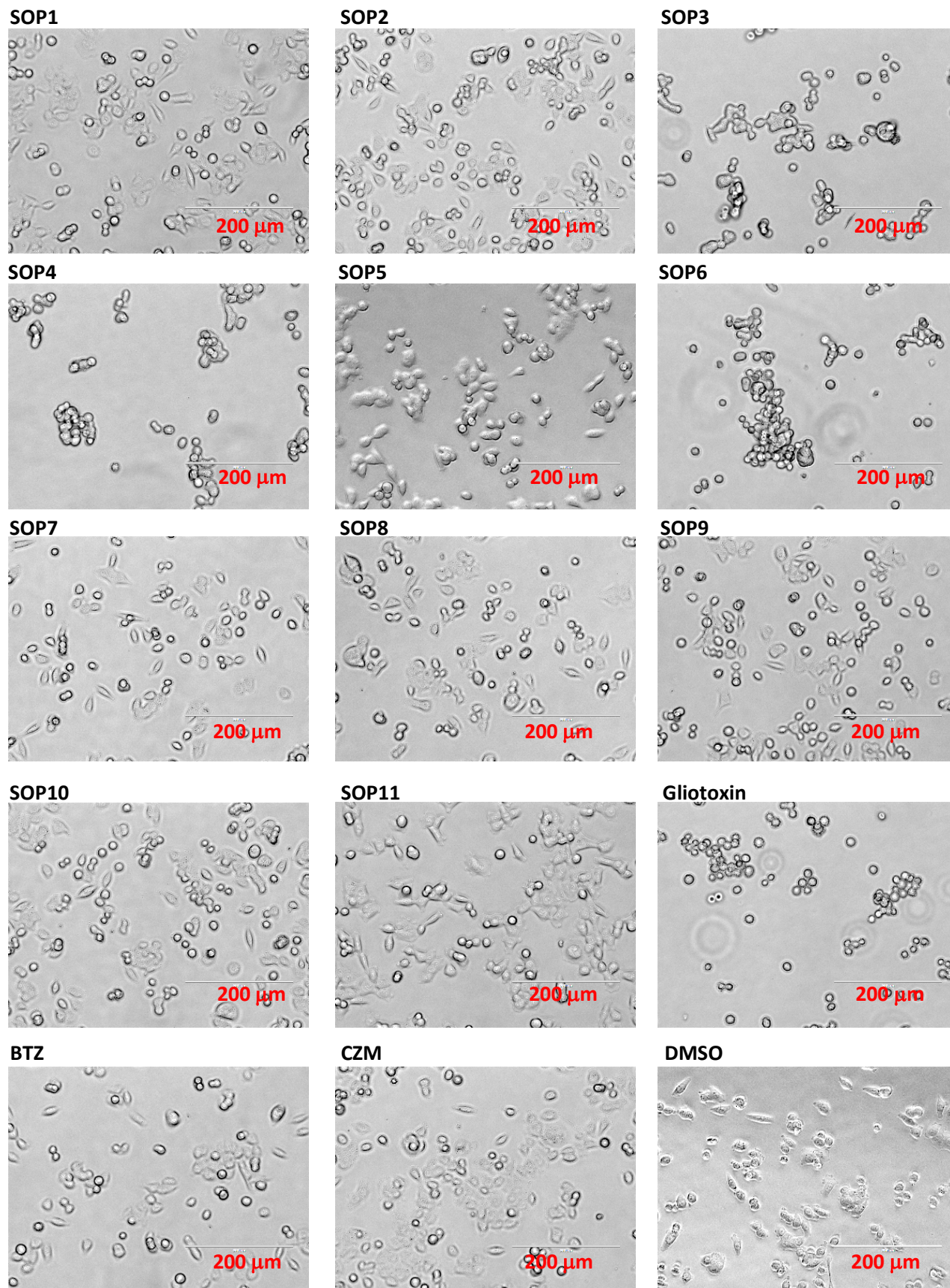


Figure S2. Effects on the cell morphology. Related to **Figure 4**. Shown are microscopic images of HCT116 cells, taken 3 hours after treatment with 10 μM of the indicated compounds except for BTZ, which was used at 1 μM.

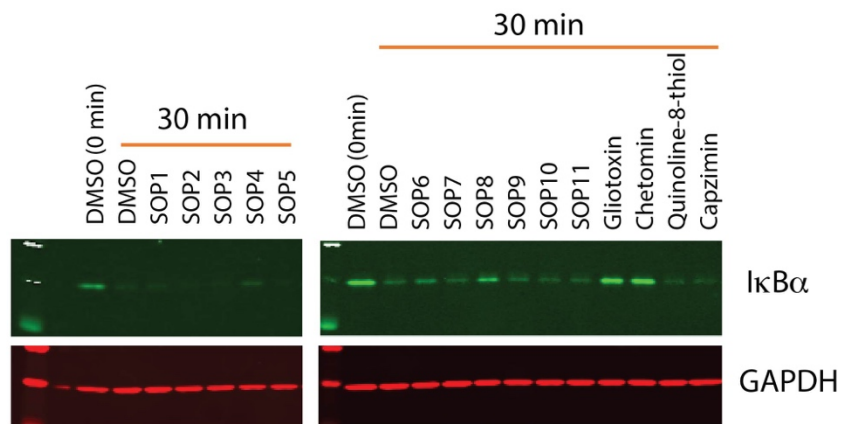


Figure S3. Screening the effects of ETPs on the degradation of IκBα in TNF-α-stimulated HeLa cells. Related to Figure 4. HeLa cells were stimulated with TNF-α for 30 mins in the presence of 1 μM compounds as indicated. Degradation of IκBα was evaluated by western blot.

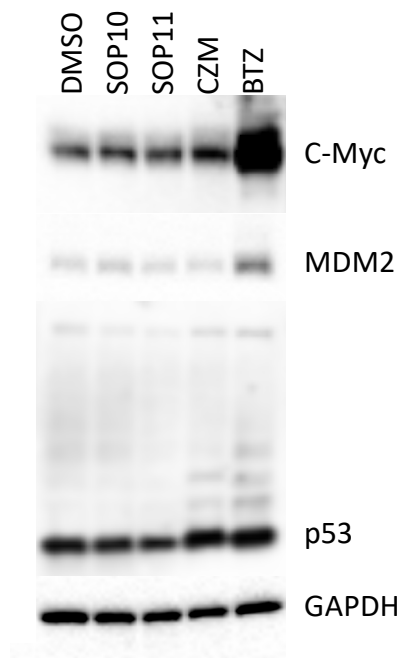


Figure S4. ETP showed little effect on stabilization of multiple proteasome substrates. Related to **Figure 4**. HCT116 cells were incubated with either ETP compounds (10 μ M), CZM (10 μ M), or BTZ (1 μ M) for 6 hours, and cell lysates were fractionated by SDS-PAGE and immunoblotted with antibodies against c-Myc, MDM2, p53 or GAPDH.

	K63 (%)	K48 (%)	K33 (%)	K29 (%)	K27 (%)	K11 (%)	K6 (%)
^{K63}UbⁿGST-Wbp2	94.13±0.28	1.69±0.06	0.34±0.03	2.13±0.03	0.04±0.01	1.49±0.16	0.19±0.02
^{K48}UbⁿGST-Wbp2	5.29±0.06	87.18±1.60	0.22±0.03	Not detected	0.03±0.00	3.78±0.27	3.52±0.67

Table S1. Mass spectrometry characterization of the polyubiquitinated GST-Wbp2. Related to Figure 1.

Reference	Structure	Rpn11 IC ₅₀ (μM)	Csn5 IC ₅₀ (μM)	AMSH IC ₅₀ (μM)	Ub ^{G76V} GFP IC ₅₀ (μM)	GI ₅₀ (μM)
SOP1		2.6±0.6	0.2±0.06	0.6±0.04	5.7±0.6	8.6±2.0
SOP2		1.1±0.3	1.2±0.3	3.2±0.2	10.7±1.6	>20
SOP3		1.3±0.4	0.4±0.1	0.5±0.04	5.0±0.8	5.7±1
SOP4		0.2±0.05	0.5±0.1	0.3±0.04	14.6±3.1	5.8±1
SOP5		3.1±1.0	0.7±0.3	1.0±0.1	8.0±1.1	>20
SOP6		3.8±1.2	2.9±0.5	2.1±0.2	4.6±1.2	1.4±0.1
SOP7		>100	>100	>100	>50	>100
SOP8		3.7±1	1.2±0.2	0.9±0.04	>20	>20
SOP9		1.9±0.4	0.9±0.3	0.4±0.03	>20	>20
SOP10		0.7±0.2	0.6±0.2	1.0±0.04	4.6±1.1	8.2±1.0
SOP11		1.3±0.3	0.6±0.2	0.9±0.1	2.7±0.7	4.7±0.5
Gliotoxin		6.9±3.4	14±3	3.6±0.6	0.4	0.3±0.04

Table S2. Summary table of the ETPs. Related to Figure 3 and Figure 5.